Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

VARIATIONS IN THE HYDROGEN-ION CONCENTRATION IN UNINOCULATED CULTURE MEDIUM

LINWOOD G. GRACE AND FLORENCE HIGHBERGER

From the Research Institute of the National Dental Association, Cleveland, Ohio

Within the last few years, the methods of adjusting the reaction of bacteriologic culture mediums have undergone considerable change. Titrating, using phenolphthalein as an indicator, has been shown to be almost as inaccurate as the older method of using litmus. A faint shade of pink was considered the neutrality point, but was difficult for even experienced workers to determine accurately this point. Further, it was almost impossible for one worker to duplicate another's results. It is possible that many discrepancies found in the results of different investigators may be due to this fact.

The newer method of adjusting the reaction by determining the hydrogen-ion concentration more nearly approaches the ideal than anything yet advanced and is fast finding its way into all bacteriologic laboratories. By this method the reactions of different batches of mediums can be more carefully controlled. So much has been written concerning this method and its advantages that any lengthy discussion would be mere repetition.

By comparing the titratable acidity as determined with phenolphthalein with the P_H value, Norton[1] and others have shown the wide variations possible in different batches of mediums when the older method was used, although it was apparently made and titrated in the same way. Carrying out the same thought along different lines Ayers, Johnson and Davis[2] found that titratable acidity, as a measure of the fermentative activity of an organism, is influenced so much by the nature and buffer content of the culture medium that this method should be supplanted by the determination of the hydrogen-ion concentration. Fennel and Fisher[3] found that it was only after using the hydrogen-ion determination method in making up their mediums that they obtained a luxuriant growth of pneumococci with any degree of consistency. Strong[4] speaks of the unsatisfactory results obtained by titrating mediums and suggests a simplified method for adjusting the reaction, based on the H-ion determination.

While making a study of the rate of acid production by Strep. viridans[5] some interesting facts concerning the broth used were brought to light.

Received for publication Feb. 1, 1920.

---

[1] Am. Jour. Pub. Health, 1919, 9, p. 190.

[2] Jour. Infect. Dis., 1918, 23, p. 290.

[3] Ibid., 1919, 25, p. 444.

[4] Jour. Am. Med. Assn., 1919, 72, p. 413.

[5] Jour. Inf. Dis., 1920, 26, p. 451.

Briefly, our experiment was this. The same strain of Strep. viridans was grown simultaneously in broth, the composition of which was the same, but the initial reaction was varied between P_H 6.4 and P_H 8.0. We then made observations on the rate of acid production with the idea of determining the reaction which favored most rapid growth.

The medium used had the following composition: Beef extract (Liebig's), 0.3%; peptone (Difco), 1%; NaCl, 0.5%; glucose, 1%. The batch was divided into 5 parts which were given the reactions of P_H 6.4, P_H 6.8, P_H 7.2, P_H 7.6 and P_H 8.0, respectively, and then autoclaved at 15 pounds' pressure for 15 minutes. The reaction was then readjusted, the mediums filtered, tubed and again autoclaved at 15 pounds' pressure for 15 minutes.

The reaction was adjusted by the simplified colorimetric method of determining the H-ion concentration as suggested by Barnett and Chapman[6] and somewhat elaborated by Norton.[1]

This procedure for making up mediums is the one usually followed, it being assumed that the change produced in the reaction by the final autoclaving would be so slight as to be disregarded.

For the experiment in question we felt, however, that it was necessary to have an exact knowledge of the H-ion concentration of the medium just before it was inoculated. Careful tests were accordingly made to determine this. We also kept uninoculated tubes of the various reactions in the incubator and noted their reaction at the conclusion of the experiment. These control tests brought out the fact that the reaction was not as stable as we had once supposed. Not only would the final autoclaving produce a change, but the reactions also varied from day to day.

In an effort to determine whether there were any consistent changes or whether the reaction would finally reach an equilibrium, we carried out four tests.

Batches of the medium were made up at different times, under conditions as nearly identical as we could make them, according to the procedure given. Some of the tubes of each reaction were put in the incubator at 37 C., some in the icebox and some allowed to stand at room temperature. Each day the P_H value of one tube from each batch was tested. Numerous checks served to show that the reaction of one tube was practically the same as that of others of that same set at that particular time.

---

[6] Ibid., 1918, 70, p. 1064.

TABLE 1

RESULTS FROM ONE TEST

1% Glucose Broth			Medium Stored in Tubes									Medium Stored in Flasks							
Initial Reaction Given Mediums	Reaction after First Autoclaving	Reaction after Final Autoclaving		Reaction after Standing One Day	Second Day	Fourth Day	Sixth Day	Seventh Day	Eighth Day	Ninth Day	Tenth Day	First Day	Second Day	Fourth Day	Sixth Day	Seventh Day	Eighth Day	Ninth Day	Tenth Dyy
PH6.4	PH6.4	PH6.4	R.	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.4	PH6.3
			Inc.	6.4	6.3	6.3	6.4	6.4	6.4	7.2	6.3	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
			I.B.	6.4	6.6	6.4	6.5	6.4	6.4	6.4	6.6	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
6.8	6.8	6.6	R.	6.8	6.8	6.8	6.8	7.2	6.5	6.6	6.8	6.8	6.8	6.8	6.8	6.6	6.6	6.6	6.6
			Inc.	6.6	6.8	6.8	6.6	6.6	6.6	6.4	6.8	6.8	6.8	6.8	6.8	6.6			
			I.B.	6.8	6.8	6.8	6.8	7.2	6.6	7.0	6.8	6.8	6.8	6.8	6.8	6.6	6.6	6.6	6.6
7.2	7.0	7.2	R.	6.6	6.3	6.3	6.3	6.3	6.4	6.4	6.5	7.1	7.2	7.0	7.0	7.0	7.0	7.0	7.2
			Inc.	6.6	6.3	6.3	6.5	6.4	6.4	6.4	6.3	6.9	6.9	6.9	6.9	6.5	6.9	6.9	6.9
			I.B.	6.5	6.4	6.3	6.3	6.4	6.4	6.3	6.3	7.1	7.2	7.0	7.0	7.0	7.0	7.0	6.9
7.6	7.2	7.3	R.	7.4	7.4	7.4	7.4	7.4	7.3	7.4	7.3	7.2	7.2	7.3	7.2	7.0	7.0	7.2	7.0
			Inc.	7.4	7.4	7.3	7.4	7.4	7.3	7.4	7.3	7.3	7.0	7.0	7.0	6.6	6.9	6.9	6.9
			I.B.	7.4	7.5	7.4	7.4	7.4	7.3	7.4	7.3	7.3	7.2	7.3	7.2	7.0	7.0	7.2	7.0
8.0	7.8	8.0	R.	7.0	7.0	7.7	7.8	7.8	7.6	7.7	7.7	8.0	7.6	7.6	7.8	7.4	7.7	7.7	7.6
			Inc.	8.0	8.0	7.7	7.8	7.8	7.6	7.7	7.7	8.0	7.6	7.6	7.8	7.3			
			I.B.	8.0	8.0	7.7	7.8	1.8	7.6	7.7	7.3	8.0	7.6	7.6	7.8	7.4	7.7	7.7	7.6

* R. = room temperature. Inc. = incubator, 37 C. I.B. = icebox.

A part of the same medium was put in flasks and kept under similar conditions in the incubator, in the icebox and at room temperature. Each day a definite amount was removed by sterile pipets and the reaction noted.

One experiment was continued for 7 days, two for 10 and one for 13 days. Table 1 gives the results obtained in one test. It shows how the reaction varied, although when compared with results obtained in the other tests there was nothing consistent about these changes. Table 2 gives the limits of change under various conditions and is taken from all the experiments.

TABLE 2

LIMITS OF CHANGE UNDER VARIOUS CONDITIONS FROM ALL EXPERIMENTS

Initial reaction given medium	P_H6.4	P_H6.8	P_H7.2	P_H7.6	P_H8.0
Limits of variation produced by autoclaving	6.3-6.9	6.6-7.2	6.9-7.4	7.2-7.8	7.4-8.0
Limits of variation in tubes at room temp.	6.4-6.8	6.5-7.2	6.3-7.2	7.1-7.9	7.6-8.0
Limits of variation in tubes in incubator	6.4-6.7	6.6-6.8	6.3-7.2	7.2-7.6	7.4-8.0
Limits of variation in tubes in icebox	6.4-6.7	6.6-7.2	6.3-7.2	7.1-7.6	7.3-8.0
Limits of variation in flasks at room temp.	6.3-6.3	6.6-7.2	7.0-7.2	7.0-7.6	7.4-8.0
Limits of variation in flasks in incubator	6.3-6.6	6.6-6.9	6.5-7.2	6.6-7.6	7.3-8.0
Limits of variation in flasks in icebox	6.3-6.6	6.6-6.9	6.9-7.4	7.3-7.6	7.4-8.0

As a final test, a batch of plain broth was treated in the same way with the results shown in table 3. While there was some variation in the reaction it will be noted that it is not nearly so great as was observed in the glucose broth. Presumably, therefore, the resulting acid formed by the breaking up of the glucose is the chief factor responsible for these changes. Amino acids formed from the peptone may also be a contributing factor.

We checked our work carefully in order that the charge of faulty technic would not be made to account for these variations. Every care was used in the preparation of the medium. The flasks and tubes were cleaned before using by boiling in soap suds, rinsing in ammonia water, then in tap water and finally in distilled water. In order to assure the constancy of the values of the colorimetric standards, checks were made by the electrometric method. These checks showed an error not exceeding 0.06.

A preliminary report of the findings was sent to a number of bacteriologic laboratories with a request that we be advised whether any similar observations had been made. Although we received a large number of replies, only a few offered any explanations or had noted such changes.

It was suggested by Professor F. G. Novy that storage of medium in tubes must lead to change, partly because of solution of alkali from the glass, partly because of absorption of CO_2 and also perhaps because of slow hydrolysis.

TABLE 3

RESULTS OF FINAL TEST WITH PLAIN BROTH

Plain Broth			Medium Stored in Tubes						Medium Stored in Flasks				
Initial Reaction Given Medium	Reaction after First Autoclaving	Reaction after Final Autoclaving	*	Reaction after Standing Two Days	Third Day	Fifth Day	Sixth Day	Seventh Day	Second Day	Third Day	Fifth Day	Sixth Day	Seventh Day
P_H 6.4	P_H 6.4	P_H 6.4	R.	P_H 6.6	P_H 6.4	P_H 6.4	P_H 6.4	P_H 6.4	P_H 6.5	P_H 6.4	P_H 6.4	P_H 6.4	P_H 6.4
			Inc.		6.4	6.4	6.4	6.4		6.4	6.4	6.4	6.4
			I.B.	6.6	6.4	6.4	6.4	6.4	6.5	6.4	6.4	6.4	6.4
6.8	6.9	7.2	R.	7.2	7.1	7.2	7.4	7.3	7.2	7.4	7.2	7.1	7.2
			Inc.		7.1	7.2	7.2	7.2		7.4	7.2	7.1	7.2
			I.B.	7.4	7.1	7.2	7.4	7.3	7.2	7.4	7.2	7.1	7.2
7.2	7.5	7.5	R.	7.5	7.4	7.5	7.5	7.5	7.5	7.5	7.4	7.4	7.4
			Inc.		7.4	7.5	7.5	7.5		7.5	7.4	7.4	7.4
			I.B.	7.5	7.4	7.5	7.5	7.5	7.5	7.5	7.4	7.4	7.4
7.6	7.6	8.0	R.	8.0	7.9	7.8	7.6	7.8	7.9	7.8	7.8	7.6	7.7
			Inc.		7.9	7.8	7.6	7.8		7.8	7.8	7.6	7.7
			I.B.	8.0	7.9	7.8	7.8	7.8	7.9	7.8	7.8	7.9	7.7
8.0	8.0	8.0	R.	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
			Inc.		8.0	8.0	8.0	8.0		8.0	8.0	8.0	8.0
			I.B.	8.0	8.0	8.0	7.8	8.0	8.0	8.0	8.0	8.0	8.0

* R. = room temperature. Inc. = incubator, 37 C. I.B. = icebox.

Such also was the opinion of Colonel F. F. Russell of the Army Medical School at Washington where observations similar to the above had been made. In his communication Colonel Russell states that changes toward the acid side have usually been attributed to the sterilization and subsequent to the absorption of CO_2. They have observed that tubes which are flamed and ampules which are sealed with a blast lamp take up an appreciable amount of CO_2 with a change in the P_H value of several tenths. Dr. F. P. Gay advised us that similar, although not identical, observations had been made at the University of California. It seems that variations were noted only in medium which was given an initial alkaline reaction.

It is probable, of course, that these changes have always occurred in mediums without our being aware of the fact. Most bacteria will grow over a very wide range. When grown in mediums they bring about changes in reaction until a final end point is reached. This end point seems to be pretty definite for certain types of organisms and has been used as a means of identification. Clark[7] discusses the diagnostic value of the final H-ion concentration in differentiating cultures of B. coli. Ayers,[8] Ayers, Johnson and Davis,[2] and Avery and Cullen[9] have all contributed to our knowledge of the end point reached by different varieties of streptococci.

As to just how important the initial reaction given culture mediums is, we are only beginning to find out, and it is quite probable that much work will have to be done before an optimum can be definitely established for each known organism or group of organisms.

As the latter question assumes greater significance, so will it grow to be more important to have a definite knowledge of the reaction of the medium just before it is inoculated. The variations that occur independent of the activities of bacteria must also be taken into account when making such studies.

It is possible that the simple method of adding normal acid or alkali solutions to mediums to give them the required degree of acidity or alkalinity may have to undergo some modifications.

[7] Jour. Biol. Chem., 1915, 22, p. 87.

[8] Jour. Bacteriol., 1916, 1, p. 84.

[9] Jour. Exper. Med., 1919, 29, p. 215.